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# Impairing Effects of Alcohol on Object Recognition

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by

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## Abstract

Two experiments investigated effects of alcohol on the ability to recognize objects. Mice explored 2 identical objects placed in an enclosed arena. Two data collection methods, open field and automated procedure, were tested to record object exploration. In experiment 1, a novel object replaced one of the objects after 1 or 24 hours, and mice spent more time exploring the novel object at both delays. In experiment 2, we administered different doses of alcohol to mice prior to the initial exploration of the objects. Object recognition testing was then conducted 24 hours later. Results indicated that the open field method provided a more reliable measure of object exploration. Mice injected with alcohol spent roughly equal amounts of time exploring both objects, indicating that they were not able to recognize the familiar object. Together, these results suggest that alcohol impairs memory acquisition. This may be because of alcohol's established effects on the hippocampus, a structure also known to have a role in object recognition memory.

# Impairing Effects of Alcohol on Object Recognition

Alcohol is generally known to impair memory in humans and animals. According to Browning, Hoffer, & Dunwiddie (1992), memory has two phases: acquisition and retrieval. Those with damage to certain brain structures are unable to encode new memories, and thus cannot retrieve them. Likewise, alcohol has been shown to have impairing effects on the acquisition phase. Memory is also associated with long-term potentiation (LTP), which is thought to be the method by which memories are formed and strengthened through neural circuits. Research on rats shows that acute alcohol doses prevent LTP from occurring (Browning et al., 1992; Givens & McMahon, 1995).

Alcohol can interfere with LTP through various mechanisms. One way is to inhibit the release of neurotransmitters necessary to transmit information through the synaptic cleft. Another way is to inhibit the neurotransmitter receptors on the postsynaptic cell. Alcohol can also strengthen the receptor for the neurotransmitter GABA, which inhibits communication between neurons (Browning et al., 1992).

To learn more about the relationship between alcohol and memory, a number of different memory tests have been used in animals. One of the more recently developed tests is the object recognition test. This test exploits the tendency of animals to prefer exploring novel objects in an environment when a familiar object is also present (Ennaceur & Delacour, 1988), and thus is not confounded by external reinforcement. Recent research indicates that alcohol intake impairs the performance of rats on the object recognition test (Ciccocioppo, Antonelli, Biondini, Perfumi, Pompei, & Massi, 2002; García-Moreno, Conejo, Capilla, García-Sánchez, Senderek, & Arias, 2002).

There has not been much research on the effects of alcohol on object recognition in mice, but Ryabinin, Miller, and Durrant (2002) showed that mice injected with a high dose of alcohol

(2.4 g/kg) did not recognize a familiar object in an object recognition task performed at a 24-hour delay. In addition, mice injected with scopolamine, a drug that blocks acetylcholine receptors, showed decreased performance on an object recognition task performed at a 3-hour delay (Dodart, Mathis, & Ungerer, 1997).

Many brain regions are likely to be involved in object recognition, but most studies have focused on the hippocampus. Lesion studies in mice and rats have revealed conflicting results with respect to the importance of the hippocampus to object recognition. For example, an intact hippocampus was necessary for mice to recognize objects after a 24-hour delay, but not after a 5-minute delay (Hammond, Tull, & Stackman, 2004). The hippocampus and/or amygdala were necessary for rats to recognize objects at a 10-minute delay, but not at less than a 2-minute delay (Mumby, Wood, & Pinel, 1992). In addition, rats with lesions of the hippocampus showed impaired performance on a memory recognition test at delays of longer than 10-minutes, but not at less than a 1-minute delay (Clark, Zola, & Squire, 2000). However, Stupien, Florian, and Roullet (2003) found that the CA3-region of the hippocampus, a region which receives sensory input from the external and internal environment and retrieves that information for working memory processes, was necessary for mice to respond to spatial changes but not to recognize a familiar object after a 24-hour delay.

Observation of the animal's exploration of objects requires a large time commitment from researchers, so experiment 1 will attempt to establish an automated procedure as a reliable data collection method. Previous research has used an open field to record animal behavior. This requires the researcher to use a video camera and/or stop watch, and it is very time-intensive to code and interpret the data. The automated procedure, which records behavior through breaks in infrared beams that are stored and analyzed through computer software, will eliminate the need for direct observation that researchers use in the open field.

The literature contains mixed results concerning the time delay between the initial exposure to the object and the subsequent test for recognition. Experiment 1 will also investigate the optimal delay to use between the initial object exploration and object recognition testing phases. Previous studies on object recognition have found that alcohol or similar drugs impair object recognition in an object recognition task at delays of 1 and 24 hours (Clark et al., 2000; Dodart et al., 1997; Hammond et al., 2004; Frick & Gresack, 2003; García-Moreno et al., 2002; Ryabinin et al., 2002; Sik, van Nieuwehuyzen, Prickaerts, & Blokland, 2003). We will also use delays of 1 and 24 hours in experiment 1, and will use the delay that yields the most significant results in the second experiment.

Once the optimal time delay and data collection method is established, experiment 2 will replicate the object recognition study by Ryabinin et al. (2002). We hope to reproduce the results that mice injected with a high dose of alcohol (2.4 g/kg) do not recognize a familiar object at a 24-hour delay. As part of our hypothesis, we anticipate that mice injected with a lower dose of alcohol (1.6 g/kg) will show less impaired performance on the object recognition task than mice injected with the highest dose. In addition, we anticipate that vehicle-treated mice will recognize the familiar object after the time delay and display normal novelty-seeking behaviors.

## General Methods

### *Subjects*

Procedures used in conducting these studies were approved by the Institutional Laboratory Animal Care and Use committee. Thirty-nine healthy 4-week-old C57BL/6 male mice, housed in small groups of 4 to 5, were used in this study (14 in experiment 1, 25 in experiment 2). Each group acclimated to the vivarium one week before conditioning began, and the colony maintained a normal 12 hour light/dark cycle (lights on at 7:00 AM). We collected all

data during the light cycle. Daily handling of mice minimized the stress associated with experimenter interaction. Each mouse was used in either experiment 1 or 2, and then euthanized.

*Apparatus*

Box habituation, initial object exploration, and object recognition testing took place in a shuttle box, measuring 40 (L) X 16 (W) X 21 (H) cm. The floor consisted of parallel stainless steel grid rods measuring 3 mm in diameter spaced 8 mm apart. For the open field, a video camera was mounted over the apparatus. An experimenter blind to the conditions scored behavior from the 10 minute video clips with a stopwatch, and recorded object exploration if the mouse's nose was within 0.5 cm from the object. For the automated procedure, 8 sets of infrared beams (4 on each side) were located on the long sides 2 cm above the floor across the entire chamber and were approximately 2 cm apart from one another. Two additional sets of infrared beams (1 on each side) were located on the wide sides 2 cm above the floor across the entire chamber and were approximately 1 cm apart from each other. Together, the 10 sets of beams created a grid to measure position and general activity of the mouse (Figure 1). The mouse had to break two of the beams that surrounded the object in order for it to be scored as object exploration.

*Procedure*

For box habituation, we used 3 (experiment 1) or 2 (experiment 2) handling sessions to reduce stress associated with injections and with the apparatus. During these sessions, mice were injected with saline and placed in the apparatus for 10 minutes on each of 2 (or 3) consecutive days. Initial object exploration started the day after box habituation, and it lasted for 10 minutes. Mice received an intraperitoneal injection of saline 10 minutes before being placed in the middle

of the apparatus. There were 2 identical objects placed on opposite sides of the apparatus, and the mice were allowed to freely explore them.

*Experiment 1*

*Methods*. For object recognition testing, 2 objects (familiar and novel) were placed on opposite sides of the apparatus. We counterbalanced the placement of the familiar and novel objects (side of the apparatus) between subjects. Mice were then placed in the middle of the apparatus and allowed to explore freely for 10 minutes. Our dependent variable was time spent with objects, measured in seconds. Lower amounts of time spent with the familiar object indicated better object recognition. For all statistical tests, we used an alpha level of .05 and analyzed data using a repeated measures ANOVA.

We used a within subjects design to examine our independent variable, which was the *data collection method*. As discussed above, we compared open field and automated procedures. All mice completed object recognition testing in an open field and automated procedure. Time spent with the objects was the dependent variable for both methods.

To examine the optimal *time delay*, 14 mice were randomly separated into 2 equal groups using a between subjects design. Our independent variable was length of delay, and object recognition testing began 1 or 24 hours after initial object exploration. Time spent with objects was measured by both data collection methods described above.

*Results*. When collapsing across both time delays and both objects, the two data collection methods yielded similar values for object exploration [$F(1, 12) = .236$, $p = .636$], as seen in Figure 2. In other words, the amount of time mice spent exploring both objects was the same independent of the data collection method. However, when considering the individual time delays and objects, there were significant differences between the two methods.

Analysis of data revealed that there was a significant interaction between data collection method and object novelty on time spent with object [$F(1, 12) = 11.364$, $p < .01$]. As seen in Figure 3, mice spent more time exploring the novel object than the familiar object, but only with the open field method. For additional comparison, Figure 4 shows the effect of time delay and object novelty on time spent with object for open field (A), and then for automated procedure (B). Mice spent more time exploring the novel object at both delays in an open field. With the automated procedure, mice did not spend significantly different amounts of time with the novel versus familiar object.

Analysis of data also revealed that there was an interaction between data collection method and time delay on time spent with object [$F(1, 12) = 3.660$, $p = .08$]. As seen in Figure 5, mice tested at 24 hours in the automated procedure spent more time exploring both objects than mice tested at 1 hour in the same procedure (Ms = 42.53 and 13.37, respectively), whereas mice tested at 24 hours in the open field spent about the same amount of time exploring objects as mice tested at 1 hour in the same procedure (Ms = 21.37 and 25.95, respectively).

*Experiment 2*

With the data collection methods and time delay tested, we wanted to look at the effects of different alcohol doses on object recognition. However, there was uncertainty surrounding the data collection methods used in experiment 1. Both methods appeared to measure the same object exploration behavior (Figure 2), but a closer look at the statistics for time delay and object novelty (Figure 4) showed that the two methods could measure something different. Therefore, we re-tested both methods in experiment 2 in the future interest of establishing the automated procedure as a reliable testing condition. Furthermore, because both delays yielded similar object recognition effects in the open field and automated procedure, and because alcohol can

remain in the bloodstream for more than 1 hour thus confounding time spent with objects, we decided to use only the 24 hour delay in experiment 2.

*Methods*. Experiment 2 was identical to experiment 1 (see methods above) except that mice received an injection of alcohol prior to object exploration. We used a between subjects design and randomly separated 25 mice into 3 groups for experiment 2. Our independent variable was *alcohol dose*, with mice receiving 0, 1.6, or 2.4 g/kg of alcohol (groups 1, 2, and 3 respectively) 10 minutes before initial object exploration. In order to keep the volume of injections equal across groups, we varied the concentration of alcohol. Each group received the following concentrations of ethanol in saline (v/v, 14 mL/kg): group 1: 0%, group 2: 13.33%, group 3: 20%. Twenty-four hours later, all groups completed the object recognition testing using both data collection methods.

*Results*. Analysis of data revealed that there was a significant interaction between data collection method and object novelty on time spent with object [$F(1, 22) = 6.099$, $p < .05$]. Mice spent more time with the novel object than the familiar object, but only with the open field method (Figure 6). There was no appreciable difference in the time spent with the novel and familiar objects in the automated procedure. This confirmed our findings from experiment 1.

The data collection methods again were not significantly different from each other [$F(1, 22) = 3.754$, $p = .066$], but since the p-value was less than .10, we concluded that the open field procedure provided a more sensitive measure of object recognition, revealing that the automated procedure is not a reliable data collection method. Therefore, we restricted our subsequent analysis to open field data only.

As seen in Figure 7, analysis of data showed that there was significant interaction for open field data between object novelty and alcohol dose on time spent with object [$F(2, 47) = 8.091$, $p < .01$]. Mice injected with either dose of alcohol (1.6 or 2.4 g/kg) spent roughly equal

amounts of time with both objects ($p > .50$), indicating an impairing effect of alcohol on object recognition. Mice injected with saline (0 g/kg) spent significantly more time with the novel object than the familiar object [$F(1, 17) = 18.806$, $p < .01$].

In addition, post-hoc analysis on the alcohol doses revealed that there was a significant difference in time spent with both objects between 0 g/kg and 1.6 g/kg, with $p < .01$. There was also a significant difference in time spent with both objects between 0 g/kg and 2.4 g/kg, with $p < .01$. However, the difference between 1.6 g/kg and 2.4 g/kg was non-significant.

## Discussion

This study confirmed the impairing effects of alcohol on object recognition memory. As other studies have suggested, this may result from alcohol's interference with memory acquisition in the hippocampus, a structure in the brain that is involved with forming new memories. Object recognition was also found at both time delays, but future studies should take into consideration the lingering effects of alcohol that could potentially exist at 1 hour.

As stated in the hypothesis, both data collection methods, open field and automated procedure, should have measured the same behavior. However, the results proved otherwise. This may be explained by the direct observation recorded by the video camera mounted over the apparatus, which revealed that some mice tended to stand still in one location, such as the corner. This tendency increased among mice that received alcohol injections. There is a possibility that mice stood still at a location close enough to the object to break two of the infrared beams, which would be counted as object exploration by the automated procedure, but the video analysis would not score it as object exploration if the mouse was facing away from the object.

This leads to the idea that mice under the influence of alcohol could have been too intoxicated to physically interact with an object, and hence unable to form memories of the

identical objects during the initial object exploration. When tested at a 24 hour delay, these mice were physically able to interact with the objects, which could substitute as an initial object exploration phase. This is confirmed by the mean object exploration times seen in the results, where mice injected with either dose of alcohol spent more time exploring both objects than mice injected with saline at a 24 hour delay.

Changes in locomotor activity involving alcohol may also account for some of the results. Ryabinin et al. (2002) found that mice injected with a dose of 1.6 g/kg spent more time exploring the novel object, and mice injected with a dose of 2.4 g/kg spent equal amounts of time exploring both objects. Our study did not find a significant difference in time spent with the objects between doses of 1.6 and 2.4 g/kg. This may be due to the bi-phasic effects of alcohol on locomotor activity. In the first 10 to 20 minutes after receiving an injection of alcohol, there is a locomotor stimulatory effect, which is then followed by a gradual inhibitory effect. Ryabinin et al. (2002) injected mice 2 minutes before initial object exploration, whereas we injected mice 10 minutes before initial object exploration. Mice in our study may have had greater exposure to the gradual inhibitory effect.

Ryabinin et al. (2002) also used 40 subjects in their pretraining injection experiment, their apparatus was slightly bigger, and their cage was placed inside a cardboard box in a dimly lit room. Our study used 25 subjects, our cage was placed inside a wooden chamber, and it was subjected to more light. In addition, their mice spent almost 3 times more time exploring both objects than our mice during object recognition testing. This may indicate that our measure of object recognition was unreliable. Our data may have been skewed, and the averages could have been biased to reflect high object exploration times of one or two subjects.

Future studies may adjust the width of the infrared beams to capture the location of the mice more accurately. Alternatively, sensors could be placed on the objects and contact with the

subject's nose or front paw could be required to record object exploration. Another possibility would be to reduce the doses of alcohol so that mice maintain mobility during initial object exploration. Another recommendation would be to use different objects, such as wheels, tubes, or climbing walls. Objects such as these are generally known to be of higher interest to mice, and could better entice an animal to interact with it.

This study was successful in linking the effects of alcohol to the acquisition phase of memory, and our findings show that alcohol interferes with object recognition memory. As mentioned before, the lower dose of alcohol (1.6 g/kg) used in our study revealed an impairing effect on object exploration, an effect not found by Ryabinin et al. (2002). This agrees with research on rats, where working memory is impaired with low doses of ethanol (Givens, 1995). As a result, our test may prove to be a more sensitive measure of object recognition.

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## Figure Captions

*Figure 1.* Graphical representation of apparatus. Dashed lines indicate location of infrared beams, and shaded boxes indicate location of objects.

*Figure 2.* Effect of data collection method on time spent with object in experiment 1. Both methods yielded similar values for object exploration.

*Figure 3.* Interaction of data collection method and object novelty on time spent with object in experiment 1. Mice explored novel object more with open field only.

*Figure 4.* Comparison of time delay and object novelty on time spent with object for open field (A) and automated procedure (B) in experiment 1. Mice explored novel object more at both delays in open field. Mice did not spend significantly different amounts of time exploring the novel and familiar object in automated procedure.

*Figure 5.* Interaction of data collection method and time delay on time spent with object in experiment 1. Mice explored both objects more at 24 hrs than at 1 hr in automated procedure. Mice spent same amount of time exploring objects at both delays in open field.

*Figure 6.* Interaction of data collection method and object novelty on time spent with object in experiment 2. Mice explored novel object more with open field only.

*Figure 7.* Interaction of object novelty and alcohol dose on time spent with object for open field in experiment 2. Both doses of alcohol impaired ability of mice to recognize familiar object at 24 hr delay.

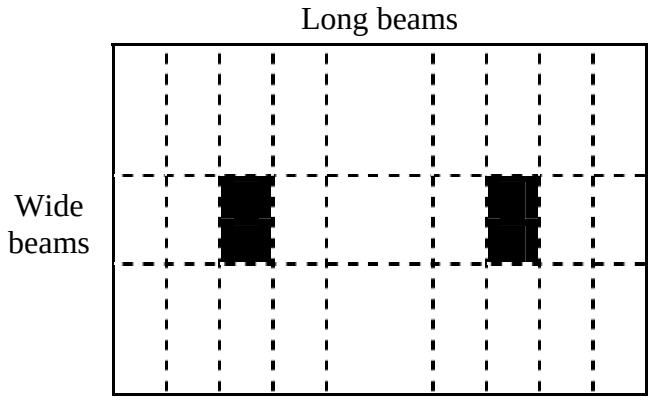



Figure 1.

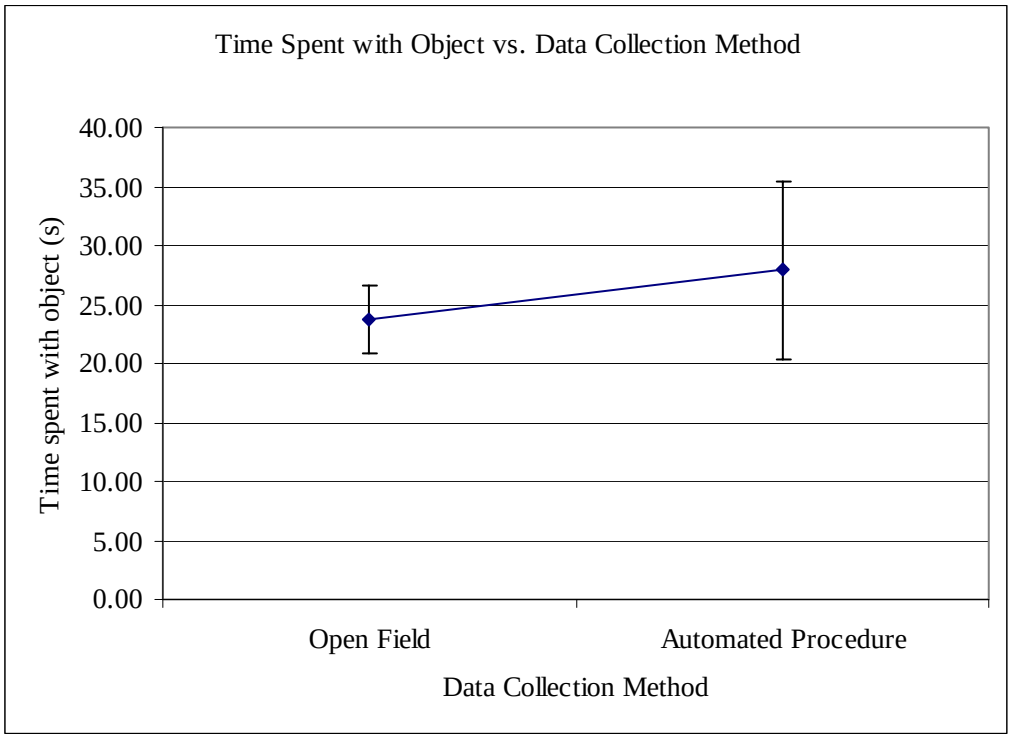
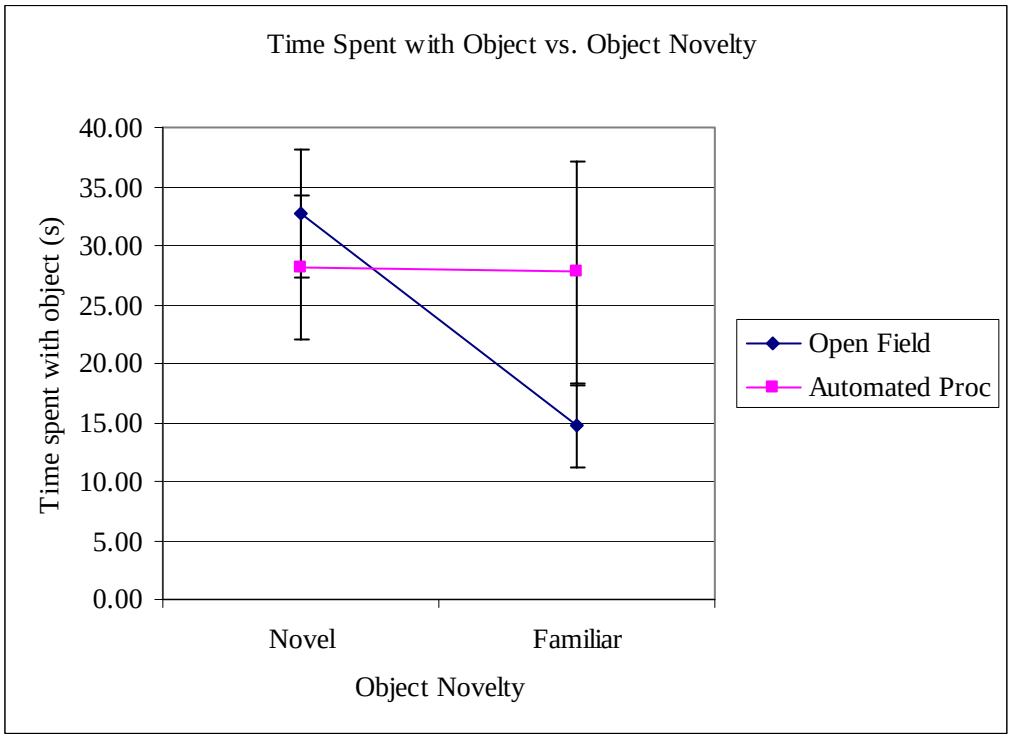

Time Spent with Object vs. Data Collection Method
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Time spent with object (s)
Open Field
Automated Procedure
Data Collection Method

Figure 2.


Time Spent with Object vs. Object Novelty
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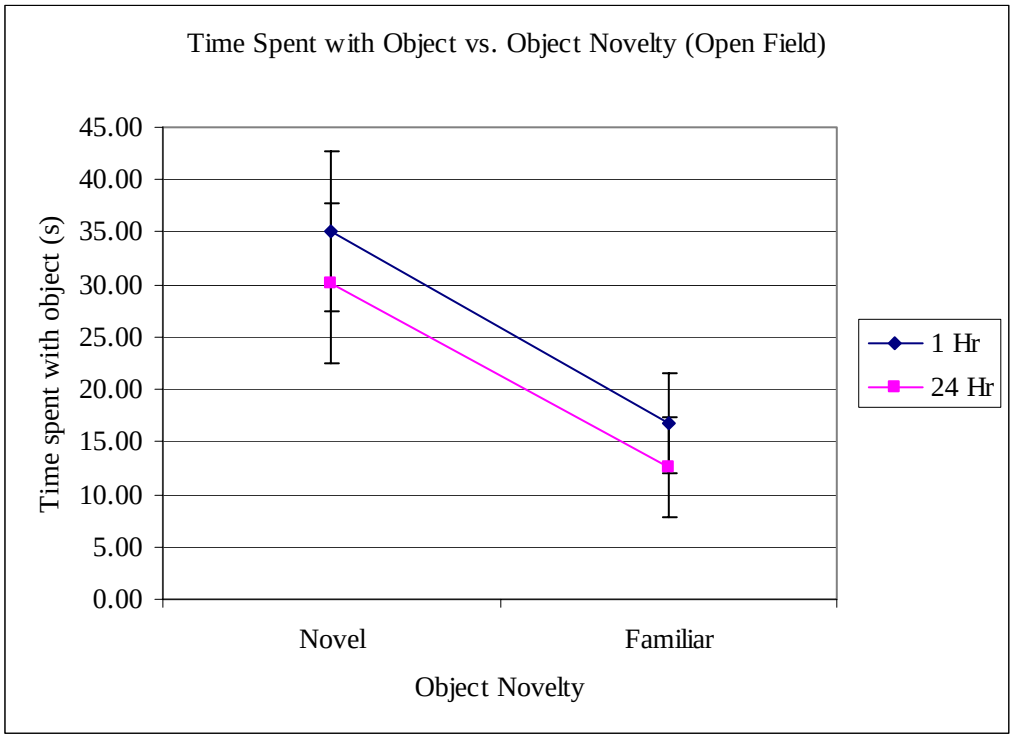
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Time spent with object (s)
Novel
Familiar
Object Novelty
Open Field
Automated Proc

Figure 3.

A.


Time Spent with Object vs. Object Novelty (Open Field)
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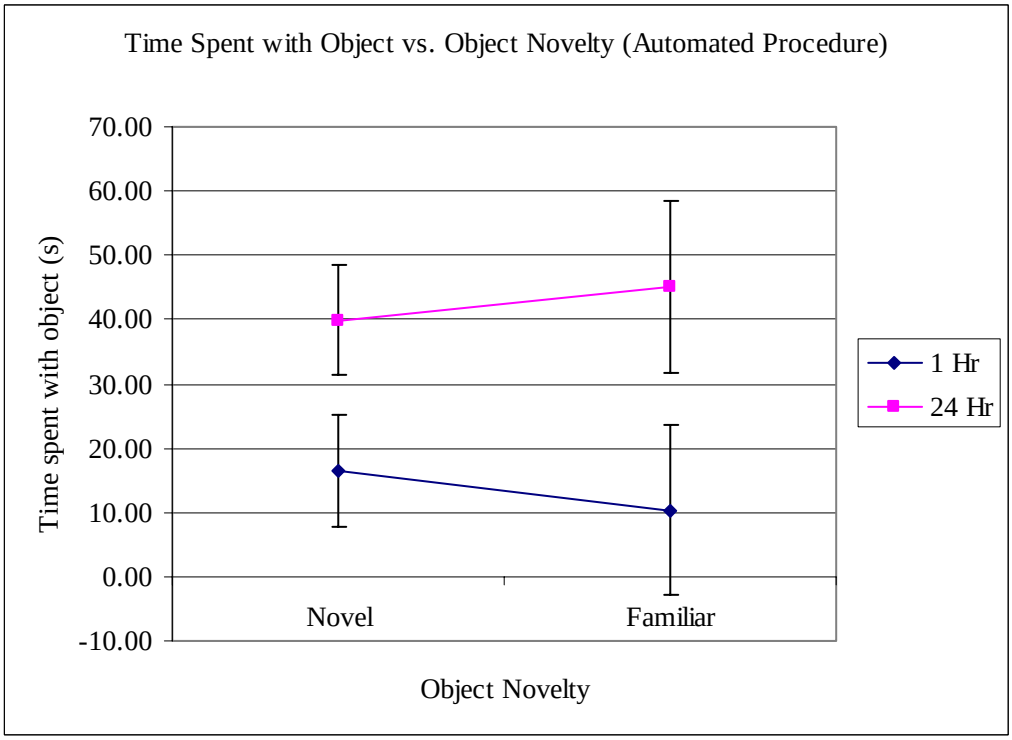
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Time spent with object (s)
Novel
Familiar
Object Novelty
1 Hr
24 Hr

B.


Time Spent with Object vs. Object Novelty (Automated Procedure)
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60.00
50.00
40.00
30.00
20.00
10.00
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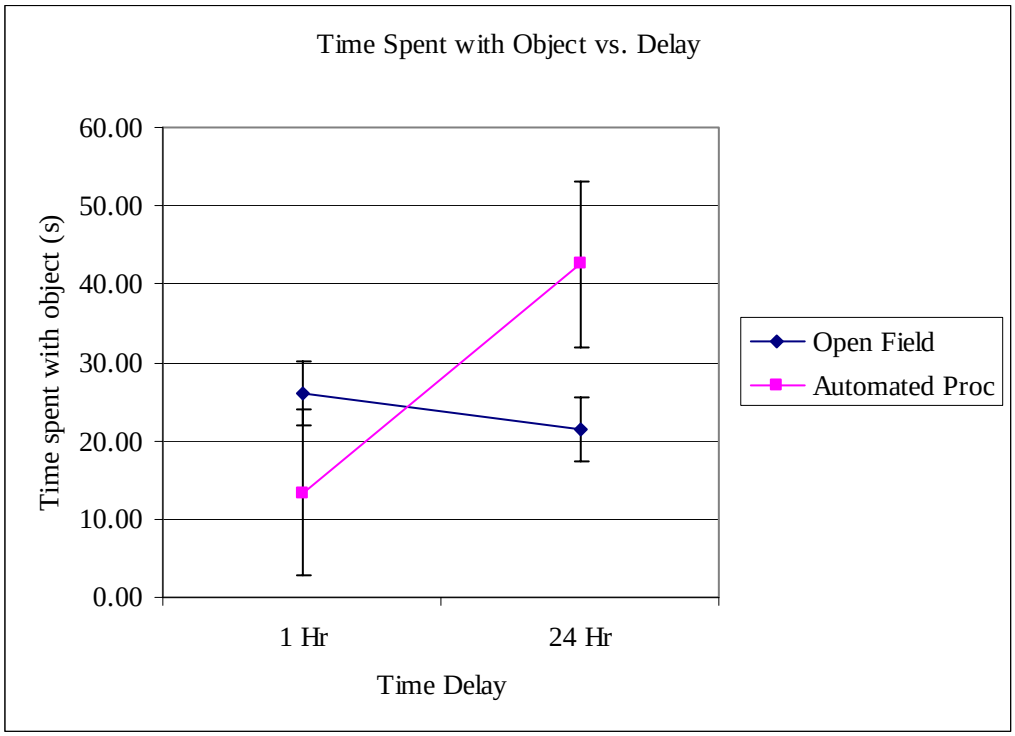
-10.00
Time spent with object (s)
Novel
Familiar
Object Novelty
1 Hr
24 Hr

Figure 4.


Time Spent with Object vs. Delay
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40.00
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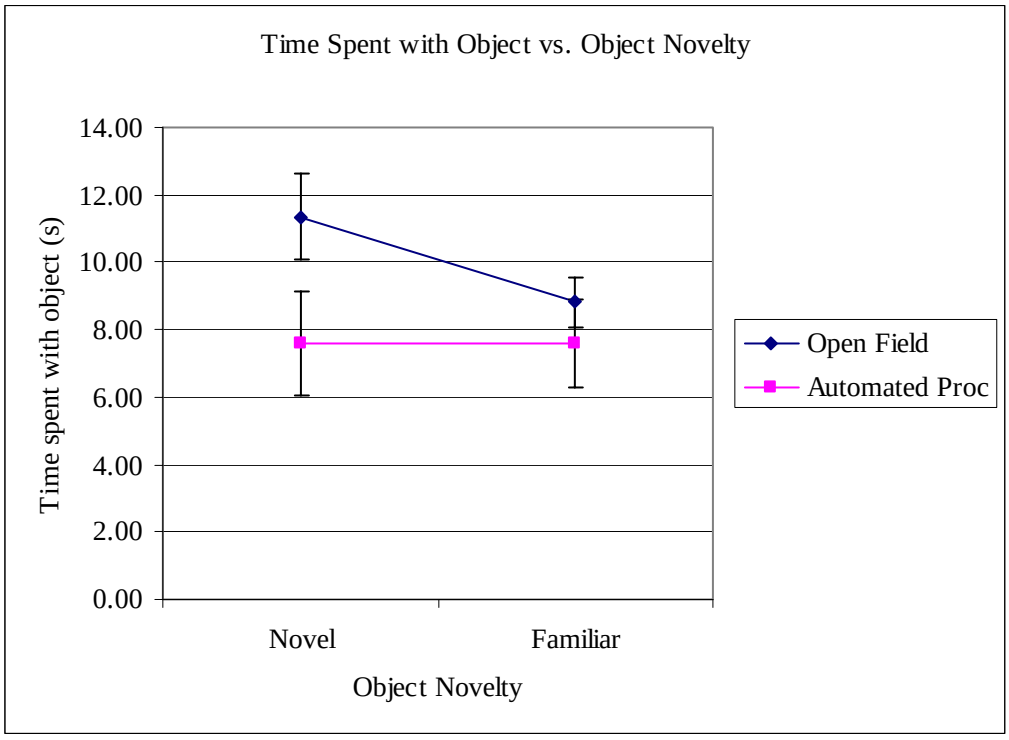
Time spent with object (s)
1 Hr
24 Hr
Time Delay
Open Field
Automated Proc

Figure 5.


Time Spent with Object vs. Object Novelty
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10.00
8.00
6.00
4.00
2.00
0.00
Time spent with object (s)
Novel
Familiar
Object Novelty
Open Field
Automated Proc

Figure 6.

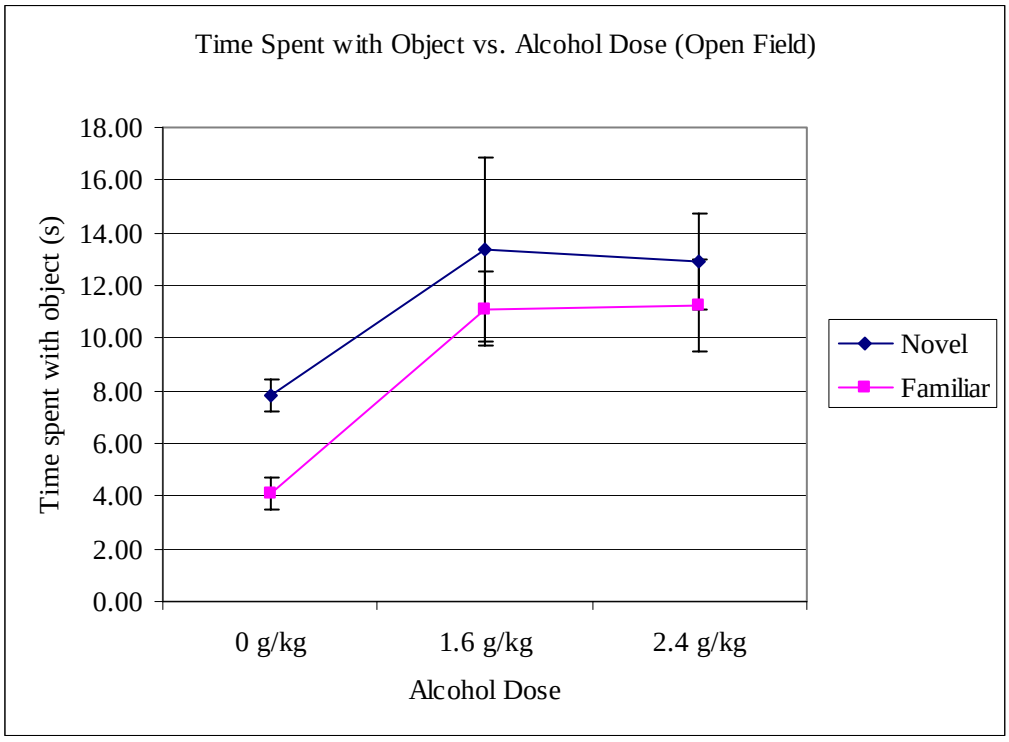
Time Spent with Object vs. Alcohol Dose (Open Field)
Time spent with object (s)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0 g/kg
1.6 g/kg
2.4 g/kg
Alcohol Dose
Novel
Familiar

Figure 7.